AKT COMPOSITIONS FOR ENHANCING SURVIVAL OF CELLS

Related Applications

This application claims priority under 35 USC §119(e) from U.S. Provisional Patent Application Serial No. 60/102,740, filed on October 2, 1998, now abandoned, entitled AKT COMPOSITIONS FOR ENHANCING SURVIVAL OF CELLS. The contents of the provisional application are hereby expressly incorporated by reference.

Field of the Invention

This invention relates to methods and compositions for the treatment of apoptotic cell-death. In particular the invention relates to Akt molecules and their use in inhibiting apoptotic cell-death of cardiomyocytes, skeletal myocytes and/or vascular endothelial cells.

Background of the Invention

Programmed cell-death (also known as apoptosis) is a form of cell-death defined by morphological and biochemical characteristics. Apoptosis is a characteristic of the normal developmental process as well as a response of cells to stress or other environmental insults. Apoptosis is characterized by membrane blebbing and retention of its integrity, cellular and cytoplasmic shrinkage, chromosome fragmentation and condensation, and endonuclease activation resulting in the characteristic 180 bp DNA ladder. During this process, the nuclear lamins are cleaved inducing their disassembly. Apoptosis does not induce an inflammatory response because cells form apoptotic bodies which are phagocytozed by neighboring cells. A number of stresses can induce apoptosis *in vitro* and *in vivo*. The administration of glucocorticoids, reduction of hormone and/or growth factor levels, chemotherapy (toxic agents), mechanical injury and DNA damage can all result in apoptosis. Apoptosis is also induced by aberrant cell cycle activity, and it can be triggered in cells that express the Fas receptor with crosslinking antibodies or the natural Fas ligand. High frequencies of apoptotic cell-death are associated in a diverse array of pathological disorders.

Akt (c-Akt) is a proto-oncogene encoding a serine-threonine kinase (Testa, J.R. and Bellacosa, A., *Leukemia Res.*, 1997, 21:1027-1031). It is the cellular homolog of the viral oncoprotein v-Akt, and is related to protein kinase-C (PKC) within the catalytic domain. However, c-Akt differs from the PKC family members by the presence of a pleckstrin homology

cells, contacting a putative inhibitory agent with the cells of the test sample under conditions to permit entry of the agent into the cell, determining a test sample index cell number, and comparing the test sample index cell number with a control index cell number of a control sample. The control sample contains cells that have been contacted with an Akt molecule under conditions to permit entry of the Akt molecule into the cells, and their index cell number is used as a reference number. The index cell number of the test sample as compared with the equivalent index cell number of the control sample is indicative of the inhibitory activity of the test agent in inhibiting death of the cells.

In one embodiment, the foregoing screening assay occurs *in vitro*. In preferred embodiments, the cells are selected from the group consisting of cardiac muscle cells (cardiomyocytes), skeletal muscle cells (skeletal myocytes) and vascular endothelial cells.

In another embodiment, the foregoing screening assay occurs *in vivo*. In preferred embodiments, the cells are cells of a subject from a tissue selected from the group consisting of myocardium, skeletal musculature and vascular endothelium.

These and other aspects of the invention, as well as various advantages and utilities, will be more apparent with reference to the detailed description of the preferred embodiments.

Brief Description of the Drawings

Figure 1. Akt expression influences myocyte viability during differentiation. **(A)** Quantification of apoptosis induced by the dominant-negative Akt expression plasmids; **(B)** Wild-type Akt promotes myocyte survival.

Figure 2. Graph showing that forced Akt expression promotes the survival of mitotic myocytes. **(A)** Myoblasts infected with Adeno-Akt express the Akt transgene as indicated by Western immunoblot analysis using either anti-Akt or anti-HA antibodies. **(B)** Anti-Akt and anti-HA immunoprecipitates from Adeno-Akt-infected myoblast cultures also contained appreciable levels of H2B kinase activity, indicating that the transgene produces functional Akt protein. **(C)** Cultures infected with Adeno-Akt displayed significantly less cell-death than control cultures.

Figure 3. Adenoviral transfection of Akt promotes the VEGF-mediated cell survival of HUVEC cultures; **(A)** the structure of replication-defective adenoviral vector expressing the Akt cDNA; **(B)** adenoviral mediated expression of Akt enhanced VEGF-induced cell survival. Data were shown as the mean +/- S.E.M. Control cultures (closed circles) received vehicle alone.

were infected with an adenoviral vector expressing β-galactosidase (Ad- β-gal), which does not affect endothelial cell viability under the conditions of our assay. As shown in Fig. 3B, adenoviral transfection of Akt markedly augmented VEGF-induced endothelial cell survival. In brief, HUVECs were cultured in 24 wells (Falcon) and infected with adenoviral vector expressing Akt (Ad-Akt) or β-galactosidase (Ad- β-gal) at a MOI of 50. After 24 hour incubation, the medium was changed to DMEM containing the indicated concentrations of VEGF. After 21 hours culture, viable cells were counted. Figure 3 Inset: HUVEC cultures were infected with Ad- β-gal (β gal) or Ad-Akt (Akt) and incubated for 24 hours. After 30 min. serum starvation, cells were treated with 1 ng/ml of VEGF for 15 min. The cell lysates were prepared and immunoprecipitated with anti-Akt antibodies. The kinase activities were measured as described in Materials and Methods. Cultures infected with Ad-Akt displayed 75 and 65% less cell-death than control cultures at 1 and 10 ng/ml, respectively, while no decrease in cell-death was detected in the cultures exposed to serum-free media in the absence of VEGF. As anticipated, adenoviral transfection of Akt also enhanced Akt kinase activity (Fig. 3B, inset). Akt immunoprecipitates prepared from Ad-Akt -infected HUVEC cultures exhibited greater kinase activity than control cultures when exposed to 1 ng/ml VEGF, the concentration of factor that produced the greatest difference in survival between test conditions. These data show that forced Akt expression can enhance the sensitivity of endothelial cells to VEGF survival signals.

Figure 4. Dominant-negative Akt inhibits IGF-1-mediated myocyte survival.

Figure 5. Overexpression of wild-type Akt facilitates myocyte survival in response to IGF-1.

Figure 6. Quantitative analysis of the *in vivo* cardioprotective effects of myrAkt.